Fate of the microbial population and the physico-chemical parameters of “Sanganel” a typical blood sausages of the Friuli, a North-East region of Italy.

Lucilla Iacumin[1], Marisa Manzano[1], Simone Stella[2] and Giuseppe Comi[1]*

[1]Department of Agricultural, Food, Animal and Environmental Sciences, Via Sondrio 2/A, 33100, Udine, Italy.

[2]Department of Health, Animal Science and Food Safety, University of Milan, via Celoria 10, 20133, Milano, Italy

*Corresponding author:

Giuseppe Comi

Department Department of Agricultural, Food, Animal and Environmental Sciences

Via Sondrio 2/A

33100 Udine, Italy

phone: +39 0432 558129; mobile: +39 338 9918561; fax: +39 0432 558130;

e-mail: giuseppe.comi@uniud.it

Abstract

In Friuli, a Northeastern region of Italy, a blood sausage called Sanganel is produced by farmers, butchers, shops, and factories. This sausage is made with pork meat, boiled blood, lard, spices, and salt. It is stored at 4 ± 2 °C and usually eaten fresh or boiled within 14 days of its manufacture. Little is known about its microbial populations and safety for consumption. The aim of this study is to characterise the microbial populations and the physico-chemical parameters of Sanganel to establish its quality and the safety of consuming it. The microbial population of Sanganel is typical of meat products, and psychrotrophic enterobacteria and lactic

acid bacteria (LAB) grow while it is stored. Enterobacteria produce total basic volatile nitrogen (TVB-N) and biogenic amines that, despite the presence of LAB, increase the pH of the sausage to approximately 6.9. Considering the concentrations of Enterobacteriaceae and TVB-N in the sausage, a shelf-life of 14 days is suggested. However, at 30 days the sausage is safe to eat and presents normal odours and flavours. In addition, boiling the sausage for 30 min before consumption eliminates the asporogenous microbial population.

Key words: Fresh Blood Sausages; Microbial Characterization; Physico-chemical parameters; Safety.

1. Introduction

Blood sausages are popular in many parts of the world. In Italy, they are mainly produced by families, particularly in areas where rural cultures still dominate. Each Italian area has its own ingredients and recipes, which differ by region, and blood sausages are a means of using every edible part of the pig. Recently, blood sausages have been rediscovered by the Italian population, but they continue to be produced by artisans and butchers using the same ingredients, recipes and technology as in the past. In other parts of the world as well, despite being widespread, blood sausages are still produced by butchers and local facilities and distributed and eaten locally (Diez et al., 2008). They are considered an ethnic product, which is one reason for their rediscovery. The European Union has promoted the protection of traditional foods from specific regions, with the aim of improving the traditional food production of rural areas and supporting local livestock production (Santos et al., 2005). In Friuli, a Northeastern region of Italy, a blood sausage called Sanganel (SBS) is produced by farmers, butchers and local factories at the level of local restaurants, taverns, and inns (called Frasche). The SBS is a traditional product, historically eaten in Friuli, frequently with brovada, a typical vegetable dish made with cabbage fermented

by pomace, with salad leaves (radicchio), or with cornmeal mush (polenta). SBS is made with bloody pork (bacon rinds and tender muscles from the pig's head, lungs, and kidneys), lard and boiled blood (boiled in water for 45 min). First, 2 kg of boiled blood, mixed with 3 kg of bloody meat and lard, are ground and mixed with salt (3.0%), pepper (0.5%), coriander, cinnamon, and ascorbic acid. The mixture is stuffed into pork bowels and stored at 4 ± 2 °C for 14 days; the SBS must be consumed fresh or boiled for 30 min. Some SBS recipes also include buckwheat flour, stale bread, pine nuts, and dried raisins. SBS processing is performed in local households in areas normally used for the butchering and processing of pigs for family consumption. SBS processing occurs after slaughter, with care taken to cleanse and disinfect the environment and tools used between one operation and the next. No data are available about the microbial and physico-chemical characteristics of SBS, and in particular, no data are available about the microbial populations and physico-chemical parameters of SBS during storage. Because SBS is made with fresh bloody meat, its microbial population likely includes *Pseudomonas* spp, Enterobacteriaceae, and Lactic acid bacteria (LAB). Morcilla de Burgos, a popular cooked blood sausage produced in the region around Burgos, in the North of Spain, and Morcela de Arroz, a popular Portuguese cooked blood sausage from Serra de Monchique (in the South of Portugal) are very similar to SBS. After cooking, Morcilla and Morcela de Arroz are contaminated by the typical aerobic microorganisms causing spoilage, predominantly *Pseudomonas*, which are introduced from handling or during the chilling step (Pereira et al., 2015; Diez et al., 2008, 2009a, b; Santos et al., 2005). Then, after packaging in a Modified Atmosphere Packaging (MAP) or vacuum packaging (VP), these microflora are replaced by homofermentative and heterofermentative LAB (Santos et al., 2005). After being cooked for approximately 1 h at 90–95 °C, air cooled to 8–10 °C, and then stored at 4 °C, the shelf-life of Morcilla is between 14 and 21 days, depending on the amount of initial contamination and the storage conditions (Santos et al., 2005). At the end of its shelf-life, the Morcilla casing is covered by a white, wet slime with a sour odour and taste, caused by LAB. In particular, the bacteria found on spoiled Morcilla

79 packaged in MAP or in VP are primarily heterofermentative LAB of the *Weissella viridescens*,
80 *Leuconostoc mesenteroides* and *Leuconostoc carnosum* species, which cause the packaging to
81 inflate, losing its vacuum seal (Santos et al., 2005).
82 SBS cannot be spoiled by LAB because it is sold or stored unpackaged at 4 ± 2 °C. The
83 aerophilic atmosphere and partial surface dehydration of the casing experienced during storage
84 delay the growth of LAB and eliminate the production of slime. Considering the absence of data
85 on the microbial and physico-chemical characteristics of SBS, the aim of this study was to
86 determine the microbial populations and physico-chemical parameters of SBS throughout its
87 shelf-life, to determine its quality and utility.
88
89 **2. Materials and Methods**
90
91 *2.1. Sampling and microbiological and physico-chemical analyses*
92 Three different lots of SBS, collected in September and November 2015 and February 2016,
93 were investigated. Each lot included 40 fresh SBS, which were produced by an artisanal
94 laboratory in the Friuli region and stored at 4 ± 2 °C for 30 days. At each time points (0, 7, 14,
95 and 30 days), 10 SBS were analysed to evaluate the microorganisms present, the physico-
96 chemical parameters and to determine the shelf life and safety of the sausages. The casings were
97 aseptically removed from each sausage and the meat was homogenized in a stomacher bag in a
98 laboratory blender (P.B.I., Italia).
99 *2.1.1. Microbial analysis*
100 Twenty-five g of the homogenate was serially diluted in stomacher bags using 225 ml of saline-
101 peptoned water (8 g/l NaCl, 1 g/l bacteriological peptone, Oxoid, Italy, 1,000 ml distilled water)
102 and homogenized in a laboratory blender (P.B.I., Italia) for 3 min. One or 0.1 ml of each serial
103 dilution was poured or spread on the following agars to evaluate the microorganisms present.
104 Total Viable Count (TVC) was determined on Plate Count Agar (PCA, Oxoid, Italy) incubated at

105 30 ºC for 48-72 h; LAB on De Man Rogosa Sharpe (MRS) agar (Oxoid, Italy) incubated at 42 ºC
106 for 48 h; yeasts and moulds on Malt Agar (MA) (Oxoid, Italy) incubated at 25 ºC for 72-96 h;
107 *Escherichia coli* on Violet Red Bile Lactose Agar (VRBLA) (Oxoid, Italy) incubated at 44 ºC
108 for 24 h; Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA) (Oxoid, Italy)
109 incubated at 37 ºC for 24 h; *Pseudomonas* spp. on *Pseudomonas* CFC Agar (Oxoid, Italy)
110 incubated at 25 °C for 48 h; Coagulase positive catalase positive cocci (CPCPC) on Baird-Parker
111 agar medium (BP, Oxoid, Italy), supplemented with egg yolk tellurite emulsion (Oxoid, Italy)
112 incubated at 35 ºC for 24-48 h and then confirmed with a coagulase test; Coagulase Negative
113 Catalase Positive Cocci (CNCPC) on Mannitol Salt Agar (MSA, Oxoid, Italy) incubated at 30
114 °C for 48 h; Enterococci on Kanamicina Aesculina Agar (KA, Oxoid, Italy) incubated at 37 °C
115 for 48 h; and Sulphite-reducing clostridia in Differential Reinforced Clostridia Medium (DRCM,
116 VWR, USA) incubated at 37 °C for 24-48 h in an anaerobic jar with a kit (gas pack anaerobic
117 system, BBL, Becton Dickinson, USA). *Salmonella* spp. was evaluated by the ISO (6579-1 2002
118 Cor.1:2004 Microbiology of food and animal feeding stuffs – Horizontal method for the
119 detection of *Salmonella* spp.) method and *Listeria monocytogenes* was also evaluated by the ISO
120 (11290-1,2:1996 Adm.1:2004 Microbiology of food and animal feeding stuffs – Horizontal
121 method for the detection of *Listeria monocytogenes*) method. Enterohemorragic *E. coli* were
122 detected by the ISO TS 13136 (EU Commission Regulation No. 209/2013, 11/03/2013; Official
123 Journal European Union, 12/03/2013, L68/19)
124
125 *2.1.2. Isolation and identification of Pseudomonas* and *Enterobacteriaceae*
126 At each sampling days of each lot, 25 colonies were randomly isolated from the *Pseudomonas*
127 CFC agar (300 colonies) and twenty-five colonies were isolated from VRBGA agar plates (300
128 colonies). The colonies were purified in PCA incubated at 30 °C for 48 h. Then, each colony was
129 subjected to gram staining and to an oxidase test. Both oxidase negative and positive strains were
130 identified by an Api system according to the manufacturer's instructions (BioMerieux, France).

131

132 *2.1.3. Physico-chemical analysis*

133 The total volatile basic nitrogen (TVB-N) was determined by the Pearson method (1976). The
134 pH values were determined directly by inserting a pH-meter probe into the homogenate
135 (Radiometer, Denmark). The water activity (Aw) was determined using a Hygromer AWVC
136 (Rotronic, Italia). The colour was determined using a Minolta Chroma meter CR-200
137 (Singapore) and a CIE Lab system. After calibration with standard white tiles, the chroma meter
138 was positioned perpendicular to the surface of the sausage, and each sample was evaluated in
139 five different positions immediately after slicing. The evaluated parameters were L*, a* and b*.
140 L* describes the white intensity or brightness, with values ranging from 0 (black) to 100 (white).
141 The a* value describes the redness (a* > 0), and b* describes the yellowness (b* > 0). Moisture
142 (A.O.A.C., 934.01 1995), salt content (Pearson, 1973), ash (A.O.A.C. 920.153 (1995), fat
143 (A.O.A.C. 960.39, 1995), protein (A.O.A.C. 928.08, 1995) were also determined. For all the
144 parameters, the final values were expressed as the respective average of ten measurements for
145 each sampling time of each lot.

146

147 *2.2. In vitro production of biogenic amines.*

148 All of the identified oxidase-negative strains were tested for the production of biogenic amines in
149 various agars according to the method proposed by Bover-Cid and Holzapfel (1999).

150

151 2.3. *Detection of biogenic amines in SBS*

152 Biogenic amine contents were determined by the method proposed by Gosetti et al., (2007).

153

154 2.4. *Volatile compounds determination*

155 At 0, 7, 14 and 30 days, ten SBS of each lot were analyzed for the presence of volatile
156 compounds, Volatile compounds were determined by SPME-GC-MS on Finnigan Trace DSQ

(Thermo Scientific Corporation, USA) with a Rtx-Wax capillary column (length 30 m x 0.25 mm id.; film thickness 0.25 µm; Restek Corporation, USA), according to the method reported in Chiesa *et al.* (2006). The volatile compounds were then identified by comparing the experimentally obtained spectra with the spectra available in the Commercial Wiley library (wiley registry 10 vers.) and a self-made library. The results represented the average of 10 samples of each of the three lots detected at each sampling time.

2.5. *Sensory evaluation*

The method used for sensory evaluation was according to Santos et al., (2005) and Kotzekidou and Bloukas (1996) modified. Ten non-professional panelists (workers in the craft factory) were asked to taste the SBS to evaluate different parameters; four additional SBS samples of each lot stored at 4 ± 2 °C were used at 0, 14 and 30 days of storage. Briefly: Sensory analysis was performed during two sessions (Santos et al. 2005). At the first session, the external appearance was evaluated; at the second session, the SBS were boiled for 30 min, cut in 1-cm thick slices and slice appearance and odour were evaluated at room temperature. External appearance, slice appearance, odour, and taste were scored on a 5-point hedonic scale as follows: 5 = excellent, 4 = good, 3 = acceptable, 2 = fair and 1 = unacceptable (Kotzekidou and Bloukas, 1996). When low scores were given, a reason was required. Unpleasant odours were evaluated on a scale where 1 corresponded to the absence of these odours or odours of a minimum intensity and 5 corresponded to odours of a maximum intensity (Santos et al., 2005).

2.4. Statistical analysis

The values of various parameters were compared using a one-way analysis of variance. The averages were compared with the Tukey's honest significant test using the StatGraphics software package from Statistical Graphics (Rockville, Maryland).

183 **3. Results**

184 The different groups of microorganisms detected in SBS are shown in Table 1. The TVC and
185 LAB concentration increased for the complete storage period and at 30 days their values were
186 about 8.5 log CFU/g. The Enterobacteriaceae also increased, from approximately 3.1 log CFU/g
187 to 8.2 log CFU/g at the end of storage. The CNCPC concentration varied at each sampling point,
188 and its concentration was about 6 log CFU/g at the end of storage. Yeast increased till 14 days,
189 then decreased, measuring approximately 5 log CFU/g at 30 days. Enterococci increased through
190 the storage reaching about 4.8 log CFU/g. The CPCPC, mould, and *Clostridia* H_2S+
191 concentrations were always less than the limit of detention of the method (< 10 CFU/g). Despite
192 the fact that CPCPC and CNCPC co-grow on MSA, it was concluded that CPCPC concentration
193 was lower than 10 CFU/g. In fact, their count on BP, the main differential agar suggested for
194 detection in food (ISO 6888-1, 10/1999), was always lower than the detection limit of the
195 method (< 10 CFU/g).

196 *E. coli* and *Pseudomonas* spp. also did not grow during storage. Enterohemorragic *E. coli,*
197 *Salmonella* spp. and *Listeria monocytogenes* were not found in any SBS samples. Three hundred
198 colonies were isolated from *Pseudomonas* selective agar and from VRBGA. After Gram staining
199 and oxidase test, the colonies were divided in two groups: oxidase negative and positive (Table
200 4). Among oxidase negative colonies, *Hafnia alvei* (45%) and *Serratia liquefaciens* (18%) were
201 the most frequently isolated strains. *Citrobacter freundii, Enterobacter cloacae, Pantoea*
202 *(Enterobacter) agglomerans,* and *Klebsiella oxytoca* were also isolated. Among oxidase positive
203 colonies, *Pseudomonas putida* (32%) and *P. fluorescens* (30%) were the most frequently isolated
204 strains. The remaining colonies were identified as *P. cepacea, P. fragi, Shewanella putrefaciens,*
205 and *Moraxella* spp.

206 Measurements of the physico-chemical parameters better demonstrated microorganism activities
207 (Table 2). The pH value was initially 6.4, and the pH increased to 6.9 at 30 days. The physico-
208 chemical parameters (Aw, Ash, Moisture, Protein, Sugar) did not change during the days of

sampling ($p > 0.05$). The Aw was about 0.96, ash about 3%, fat about 34%, moisture about 47%, protein about 14% and sugar about 1.5 %. The SBS recipe is standardized and has not changed. It is always respected during each lot of production, because the producers retain that changing in the ingredient ratioes produce differences in flavor and odor. Consequently no physico-chemical differences can be observed among the lots (data not shown).

The TVB-N concentration increased from an initial value of 15 mg N/100 g to 52 mg N/100 g fresh matter at the end of the storage period (Table 3). Concentrations of histamine (HIS), putrescine (PUT), cadaverine (CAD), spermine (SPR), and spermidine (SPD) increased throughout storage but were always less than 15 mg/kg; conversely, tyramine (TYR) was never found in any samples. The HIS concentration was less than the limit proposed by REG. EEC 2073 (15/11/2005, L 338/1) in all samples tested (Table 3). All isolates of the oxidase negative strains exhibited the ability to decarboxylase amino acids *in vitro* (Table 4).

The colour of the SBS did not change over time, and there were no significant differences in the levels of the L*, a*, and b* parameters ($p > 0.05$) among the samples of all the lots (Table 2).

The volatile compounds (VOCs) analysis was performed on SBS at different times of storaging. The data of table 5 represent the means and the standard deviations of all the obtained results. The identification of compounds was performed either by comparison of retention times either by comparison of the spectra obtained by the mass spectrometer with those stored in the tool library and the library Wiley. The content of these substances was determined proportionally from the internal standard (ethyl-propionate). To better interpret the results obtained from the analysis of the headspace, the 57 substances identified were split into 8 classes: hydrocarbons (2), aldehydes (10), esters (10), alcohols (10), sulfur compounds (2), terpenes (20), ketones (2), carboxylic acids (1). No differences were observed among 0, 7, 14 storaged days ($p > 0.05$; data not shown). The concentrations of volatile compounds increased between the first 14 days and the 30 day of storaging. However only 9 out 57 compounds presented significative differences ($p < 0.05$).

Considering the parameters (External and Slice appearance, Odour, Taste, Unpleasant odour), the sensory evaluations established that no significant differences existed among SBS stored for 0, 14, and 30 days ($p > 0.05$). The 10 panelists reached similar results. All of the SBS samples were acceptable, and abnormal odours or flavours were not perceived by the panelists (Table 6).

4. Discussion

The Sanganel (SBS) is a blood sausage produced in Friuli, a Northeastern region of Italy. It is made by craft factories, local butchers, meat shops and sometimes also by individual families. It is a fresh sausage made with fresh bloody meat, fat, boiled blood, and additives, and stuffed into natural casings. After stuffing, the SBS is ready to eat, but it can also be boiled and sliced before consuming. It does not require ripening. However, its shelf-life is 14 days when stored at 4 ± 2 °C. Little is known about the microbial populations that are initially present in the sausage and grow during storage. The initial microbial population depends on parameters that usually permit contamination, such as which forms of microorganisms or bacterial spores survive the heat treatment of the blood, on the raw bloody meat and fat, and the handling of the product during and after processing (Cattaneo et al., 2003; Ridell and Korkeala, 1997; Korkeala et al., 1987). The initial bacterial populations found in SBS include *Pseudomonas* and Enterobacteriaceae, the typical microbial population of meat (Ercolini et al., 2006; Ridell and Korkeala, 1997). *Pseudomonas* strains did not prevail over the enterobacteria at time 0, but after 7 days of storage they were completely replaced by psychrotrophic enterobacteria and LAB.

In Morcilla and Morcela, the aerobic microbial population and *Pseudomonas* grew in samples without packaging or in air packaging (Pereira et al., 2015; Santos et al., 2005). LAB, derived from the contamination during cooling and manipulation, grew in Morcilla and Morcela stored in air, in VP and in MAP (Diez et al., 2008, 2009a,b; Pereira et al., 2015). In particulary, in Morcilla stored in air, LAB, pseudomonads, yeast and mould significantly increased during cold

storage ($p < 0.05$) and after 27 days the number of pseudomonads was higher than 6 log CFU/g and slightly higher than counts for LAB, yeast and mould (Santos et al., 2005). Moreover enterobacteria significantly grew but only at the level of 5 log CFU/g on day 49 (Santos et al., 2005). Consequently the main microbial groups that grow in Morcilla stored in air are totally different than those of SBS. In SBS samples stored in air, LAB and enterobacteria predominate over aerobic bacteria such as pseudomonads, vice versa in Morcilla samples stored in air, pseudomonas and psychrotrophs bacteria predominate (Santos et al., 2005).

Actually, the microbial populations present in SBS likely changed due to a lack of oxygen caused by the growth of aerobic microorganisms over the first days after casing. However, its pH does not decrease because the enterobacteria grow along with LAB and produce TVB-N and biogenic amines. Conversely, both in Morcilla/Morcela and in traditional sausages, LAB largely predominate and eliminate sensory spoilage via a drastic decrease in pH (Dalla Santa et al., 2014; Comi and Iacumin, 2013; Comi et al., 2005; Samelis et al., 2000; Korkeala and Bjorkroth, 1997; Korkeala et al., 1987; von Holy et al., 1992). The pH of SBS increased over 30 days of storage, and the difference among the values observed at various sampling times are significant ($p < 0.05$). The pH values of SBS are not agreed with those of Morcilla and Morcela, in which the inhibitory effect observed on yeasts, *Pseudomonas* and enterobacteria is due to the LAB growth, to the presence of carbon dioxide, and to the VP or MAP packaging process (Pereira et al., 2015; Santos et al., 2005). Carbon dioxide, in particular, delays the growth of homofermentative LAB, responsible for the decrease in pH and favouring heterofermentative LAB such as *Weissella viridescens*, *Leuconostoc mesenteroides*, and *Leuconostoc carnosum* species, which produce fewer organic acids than homofermentative LAB (Santos et al., 2005; Ahvenainen et al., 1990; Blickstad and Molin, 1983). The inhibitory effects of CO_2 have a stronger effect on Gram positive than on Gram negative bacteria (Bell et al., 1995; Jeremiah et al., 1994; Gill and Penney, 1988). However, in sausages and meat products, the growth of heterofermentative LAB is delayed because they produce changes in sensory characteristics,

286 discoloration, sour odours and tastes, and exudates (Comi and Iacumin, 2013; Ahvenainen et
287 al.,1990). SBS is not protected by MAP or VP packaging and consequently does not support the
288 growth of heterofermentative LAB. Thus, in the SBS tested, no abnormal colours, odours, or
289 flavours were observed, as it was corroborated by sensorial and physico-chemical analysis. Only
290 a small amount of moisture is lost because the traditional method of SBS distribution does not
291 include MAP or VP packaging. The drying effect observed during cold storage does not cause
292 weight loss as it does in Morcilla and Morcela stored in air (Santos et al., 2005; Diez et al.,
293 2009a, b). The reduction in moisture resulting from the storage of SBS is small, as also
294 demonstrated by the Aw values during storage. No significant differences were observed among
295 the Aw and moisture values at 0 through 30 days ($p > 0.05$). This lack of moisture loss is
296 unexpected and is due to the presence of boiled blood and in some cases to the buckwheat flour.
297 In Morcela, moisture loss is limited by the presence of blood and rice, which bind to water
298 (Pereira et al., 2015), although the effect of blood on the water retention capacity of the sausage
299 can vary (Jarmoluk and Pietrasik, 2003). Conversely, Morcilla dehydrates when stored in air,
300 and it was not acceptable after 12 days because of the drying surface (Santos et al., 2005).
301 Enterobacteriaceae grow from 7 to 30 days, and their continuous growth is the result of their
302 anaerobic characteristics. Several authors (Pereira et al., 2015; Santos et al., 2005) have
303 demonstrated similar Enterobacteriaceae and *Pseudomonas* behaviours in Morcilla and in
304 Morcela, respectively, after MAP or VP packaging. However, they attribute the observed
305 decrease in *Pseudomonas* to MAP packaging and the behaviour of Enterobacteriaceae to either
306 packaging or to the psychrotrophic and anaerobic characteristics of Enterobacteriaceae (Pereira
307 et al., 2015). Different strains of Enterobacteriaceae are able to grow at 4 °C, and at this
308 temperature *Hafnia alvei* can be isolated most frequently, as found in this study and in previous
309 studies by Ridell and Korkeala (1997). Other Enterobacteriaceae strains are also capable of
310 growing at refrigerated temperatures. *S. liquefaciens* can growth at 1.7 °C; *P. agglomerans, E.*
311 *cloacae, K. oxytoca,* and *C. freundii* have a minimum growth temperature of approximately 2-3

°C (Ridell and Korkeala, 1997). Enterobacteriaceae strains can demonstrate large variations in their minimum growth temperatures, with heterogeneity existing even within one species (Ridell and Korkeala, 1997; Niemelä et al., 1983). In SBS, the oxygen depletion occurring during storage affects the *Pseudomonas* that remain at low levels; in particular, their concentrations decrease at 14 and 30 days of storage. Conversely, either the absence of package or the oxygen inside the product support the growth of Enterobacteriaceae, which are the primary microorganisms responsible for spoilage, resulting in the development of TVB-N and biogenic amines. SBS maintains acceptable TVB-N values within 14 days, because they are less than 35 mg N/100 g. This value is recognized as the acceptable limit for fresh meat products, as suggested for fish by EEC REG. 853/05, EEC 854/05 and EEC 2074/05. The TVB-N concentration of 52 mg N/100 at 30 days is typical of spoiled products. TVB-N can also be produced by LAB. Because of the absence of added sugars in SBS, it is possible that LAB could metabolize proteins and amino acids and produced volatile compounds typical of spoilage. Various authors (Comi and Iacumin, 2013; Seefeldt and Weimer, 2000) have demonstrated that the high pH and lack of natural or added sugars in sausages induce LAB to metabolize proteins via amino acid metabolism and produce ammonia and other abnormal odours. From 14 to 30 days, SBS supports the growth of LAB and Enterobacteriaceae, but the effects of these microorganisms on sensory characteristics are minimal. Enterobacteriaceae are responsible for the production of biogenic amines. Biogenic amines are common in fermented meat and food (Gardini et al., 2008; Roig-Sagués et al., 1999;), and can cause a loss of quality in raw and fresh meat (Hernàndez-Jover et al., 1997). In SBS, only HIS and CAD were present from the first sampling time point (Time 0). PUT, SP, and SPD were found after 14 days of storage, while TYR was never found. However, the concentrations of all biogenic amines detected were low and do not represent a problem for consumers. At 30 days, the HIS concentration was approximately 10 mg/kg, an acceptable value for a meat or fish product, as EEC 2073/05 imposes a limit of 100 mg/kg. Biogenic amines were also found by previous studies in Spanish

and Italian sausages (Gardini et al., 2008; Roig-Sagués et al., 1999; Hernandez-Jover et al., 1996,1997). Data from these studies do not agree with ours because the concentrations of biogenic amines, they measured, were 100 and sometimes as much as 600 mg/kg. The production of biogenic amines depends on LAB, Enterobacteriaceae, and Enterococci (Buňková et al., 2009; Pircher et al., 2007; Suzzi and Gardini 2003). In SBS, the primary producers of biogenic amines are Enterobacteriaceae, due to their high concentrations. LAB could not be responsible for the production of biogenic amines because TYR was not found, and in sausages the presence of TYR can be only the result of LAB activity because LAB possess the tyrosine decarboxylase enzyme (Buňková et al., 2009; Pircher et al., 2007; Suzzi and Gardini, 2003). Gram negative bacteria (enterobacteriaceae and *Pseudomonas*) are the major PUT producers, because they can metabolize ornithine and arginine, which are converted into PUT (Shah and Swiatlo, 2008). However, also LAB produce PUT by agmatine deiminase pathway, after the decarboxylation of arginine (Lucas et al., 2007; Ladero et al., 2012). EFSA (2011) report showed that PUT concentration was higher in fish sauce (median 82 mg/kg), cheese product (median <50 mg/kg) than in meat product (median 26.9 mg/kg in sausages).

Enterococci also have a limited effect on the production of biogenic amines because their concentrations remained low until 30 days of storage and because of the absence of TYR, a typical characteristic product of Enterococci (Ladero et al., 2012; Marcobal et al., 2011; Gardini et al., 2008). Consequently, in SBS only Enterobacteriaceae seem to be responsible for the production of biogenic amines, particularly HIS, as found in previous studies (Gardini et al., 2008; Suzzi and Gardini, 2003; Gonzales-Fernandez et al., 2003). After the autolysis of Enterobacteriaceae, decarboxylase enzymes continue to produce biogenic amines in food products (Rossi et al., 2011; Kanki et al., 2007). A lack of TYR demonstrates the safety of a product for both traditional consumers and for patients undergoing classical monoamine oxidase inhibitor treatment considering that 6 mg of TYR has been established as the threshold for symptoms (McCabe, 1986). The presence of a TVB-N concentration up to the limit imposed by

364 the EEC for fish products and the presence of biogenic amines did not affect the sensory
365 properties or the SBS safety. SBS can be considered safe because no pathogenic microorganisms
366 were found after 30 days and SBS must be boiled before eating. The time (30 min) and the
367 temperature of boiling will eliminate the microflora responsible for spoilage and health risk.
368 However, because at 30 days the TVB-N concentration is about 52.5 mg N/100 and a food with
369 that TVB-N concentration is considered spoiled, the shelf-life of SBS should be limited to 14
370 days. At this time, all of the microbial and physico-chemical parameters of SBS are adequate and
371 reflect typical healthy and sensorial values of edible foods. Therefore, the shelf-life of SBS is
372 shorter than that of similar products such as Morcilla de Burgos and Morcela de Arroz. This is
373 probably due to the different technologies used in the production of both the products, which are
374 cooked before packaging and storage. Cooking eliminates many of the asporogenous
375 microorganisms responsible for spoilage. In addition, Morcilla and Morcela are Air, VP or MAP
376 packaged and stored at 4 ± 2 °C. Sensory analysis showed that the shelf life of Morcilla stored in
377 air did not exceed 17 days, while samples packed under vacuum and in MAP (30% CO_2) were
378 acceptable until 22 days or more of storage (Santos et al., 2005). In particular, despite Morcilla
379 storaged in air was cooked before selling, its appearance was not acceptable after 12 days due to
380 the drying surface. In addition, after 17 days the panel judges detected slightly putrid aroma,
381 mouldy taste and dehydration (Santos et al., 2005). The SBS is not cooked after production and
382 is sold unpackaged, so the microorganisms responsible for spoilage remain viable and produce
383 spoilage during the first week of storage. Asporogenous microorganisms are eliminated only by
384 boiling before eating. Because Morcilla and Morcela are popular, produced on a large scale, and
385 sold and eaten in many regions of Spain and Portugal, local facilities use additional technologies
386 to improve their shelf life. Over the last few decades, high pressure treatment, pasteurization and
387 MAP packaging have been demonstrated to be effective at extending the shelf life of Morcilla de
388 Burgos (Diez et al., 2008, 2009a, b). MAP, with a concentration of 50% of CO_2 or higher is
389 recommended to preserve Morcilla de Burgos for long periods. This product was found to be

390 sensorially acceptable after 32 days of storage in packaging with 50% and 80% CO_2, because it
391 inhibites LAB spoilage, without affecting sensory properties (Santos et al., 2005). On the
392 contrary SBS is produced locally in Friuli and remains a traditional food, so no technologies are
393 used to extend its shelf life. SBS is produced during the late fall and winter and is usually eaten
394 fresh or boiled within 14 days.
395 Fifty-seven VOCs were detected in the tested SDS and only 9 significantly increased from 14 to
396 30 days of storaging. Several of the compounds found were ascribed to the amino acid
397 metabolism of LAB and enterobacteria, to oxidation and auto-oxidation of lipids (Comi et al.,
398 2016; Montel *et al*. 1998) and to endogenous reactions that occur during cooking (Mottram,
399 1998). In particular great part of the compounds are related to added spices, bacterial activity and
400 fresh meat and the cooked blood used as ingredient. Hydrocarbons and ketones are the less
401 VOCs present. It was supposed that they could be transformed into aldehydes. This was
402 confirmed by the high concentration of aldehydes. In this study only two ketons were detected
403 Hydrocarbons are transformed into aldehydes and ketones, and this was confirmed by the higher
404 concentration of these compounds in the spoiled dry cured ham.
405 Among the organic acid only acetic acid was detected and may be due to the degrading activity
406 of LAB (Comi et al., 2016). Acetic acid can originate from the metabolism of triglycerides and
407 phospholipids or from the degradation of lipids by the activity of lipolytic enzymes (Shahidi et
408 al., 1986). Organic acids with more than two or three carbon atoms were not detected. Great
409 values of esters were present and originate from the activity of enterobacteria, *Pseudomonas*,
410 LAB, CNCPC and other bacteria (Chiesa *et al*. 2006; Comi et al., 2016).
411 Ethanol was the main alcohol produced. Alcohols can be derived from bacterial fermentation,
412 from aldehydes reduction, sugar fermentation, oxidative decomposition of lipids and Strecker
413 degradation of amino acids (Ardò, 2006). In particular, the higher alcohols could be derived
414 from bacterial conversion products of leucine, valine and phenylalanine. Aldehydes can originate
415 from either fermentation or from the oxidation of unsaturated fatty acids (hexanal, etc.). Usually,

the concentrations of aldehydes increase due to the fermentative activity of the starter bacteria in sausages or as a result of degradation during Strecker amino acid synthesis in other meat products that do not involve bacterial fermentation (Comi et al., 2016). Among the aromatic hydrocarbons (terpenes), twenty compounds were identified. They are typically found in raw materials and probably originate from various contaminations in animal feedstuffs and spices (nutmeg, black pepper), considering the fact that they are found in plants (Van Straten 1977; Comi et al., 2016) eaten by animals. For these reasons, there were not a large difference among the terpenes concentrations of SDS of all the investigated times. Only 2 sulfur compounds were detected, they could derive from the degradation of sulphur containing amino acids, such as cysteine and methionine, or from garlic that has been added during the preparation of the meat mixture (Dainty, 1996). Finally pyrazine compounds were not detected, despite a cooked ingredient (blood) is part of the recipe. In fact they derive from the cooking process (Mottram, 1998).

The differences in the concentrations of VOCs between the products storaged at 0,7,14 days and 30 days did not influence the off-odor as demonstrated by the sensorial analysis

5. Conclusion

Despite the fact that at 30 days the TVB-N values exceeded the limit of acceptability, the sensorial analysis demonstrated that SBS remained acceptable until this date. The non-professional panelists did not perceive any differences among the samples ($p > 0.05$) analysed at each established time (days 0, 14, 30) and any strong sour odour or taste in any sample. However it was suggested that product must be eaten within 14 days, as justified by low concentrations of TVB-N and biogenic amines, although SBS could be also edible and safe until 30 days of storage.

References

442 Ahvenainen, R., Kivikataja, R. L., Skytta E., 1990. Factors affecting the shelf-life of gas and
443 vacuum-packed cooked meat products. Part II: Vienna sausages. Lebensmittel-Wissenschaft
444 Tech. 23, 130–138.

445 A.O.A.C. (1995) Official Methods of Analysis. 16th Edition, AOAC, Alington, USA.

446 Ardò, Y., 2006. Flavour formation by amino acid catabolism. Biotechn. Adv. 24, 238-242.

447 Bell, R. G., Penney, N., Gilbert, K. V., Moorhead, S. M., Scott, S. M., 1995. The chilled storage
448 life and retail display performance of vacuum and carbon dioxide packed hot deboned beef
449 striploins. Meat Sci. 42, 371–386.

450 Blickstad, E., Molin, G., 1983. The microbial flora of smoked pork loin and frankfurter sausage
451 stored in different gas atmospheres at 4 °C. J. Appl. Bacteriol. 54, 45–56.

452 Bover-Cid, S., Holzapfel, W. H., 1999. Improved screening procedure for biogenic amine
453 production by lactic acid bacteria. Int. J. Food Microbiol. 53, 33-41.

454 Buňková, L., Buňka, F., Hlobilová, M., Vaňáyková, Z., Nováková, D., Dráb, V., 2009.
455 Tyramine production of technological important strains of *Lactobacillus, Lactococcus* and
456 *Streptococcus.* Eur. Food Res. Techn. 229 (3), 533-538.

457 Cattaneo, P., Stella, L. S., Pensatene, A., Cantoni, C., 2003. Alterazioni di salumi preparati con
458 carni suine DFD. Arch. Vet. It., 54 (1/2), 11-20.

459 Chiesa, L., Duchini, M., Iacumin, L., Boscolo, D., Comi, G. & Cantoni, C. (2006).
460 Caratteristiche chimiche e batteriologiche di salami della Lomellina. *Archivio Veterinario*
461 *Italiano* **57** (5) 209-224.

462 Comi, G., Andyanto, D., Manzano, M., Iacumin, L., 2016. *Lactococcus lactis* and *Lactobacillus*
463 *sakei* as bio-protective culture to eliminate *Leuconostoc mesenteroides* spoilage and improve the
464 shelf life and sensorial characteristics of commercial cooked bacon. Food Microbiol. 58, 16-22.

Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., Cocolin, L., 2005. Characterisation of Natural Fermented Sausages Produced in the North East of Italy. Meat Sci. 69, 381-392.

Comi, G., Iacumin, L., 2013. Microbial spoilage of traditional dry sausages produced in small-scale facilities in Friuli, a northeastern region of Italy. Acta Alim. 42 (3), 390-399.

Dainty, R. H., 1996. Chemical/biochemical detection spoilage. Int. J. Food Microbiol. *33*, 19-34.

Dalla Santa, O. R., de Macedo, R. E. F., Dalla Santa, H. S., Zanette, C. M., de Freitas, R. J. S., Terra, N. N., 2014. Use of starter cultures isolated from native microbiota of artisanal sausage in the production of Italian sausage. Food Science and Technology, Food Sci. Technol, Campinas, 34(4): 780-786,

Diez, A.M., Santos, E.M., Jaime, I., Rovira, J., 2008. Application of organic acid salts and high-pressure treatments to improve the preservation of blood sausage. Food Microbiol. 25, 154–161.

Diez, A. M., Santos, E. M., Jaime, I., Rovira, J., 2009a. Effectiveness of combined preservation methods to extend the shelf life of Morcilla de Burgos. Meat Sci. 81, 171–177.

Diez, A.M., Björkroth, J., Jaime, I., Rovira, J., 2009b. Microbial, sensory and volatile changes during the anaerobic cold storage of morcilla de Burgos previously inoculated with *Weissella viridescens* and *Leuconostoc mesenteroides*. Int. J. Food Microbiol. 131, 168–177.

EFSA. Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA J. 2011, 9, 2393−2486.

Ercolini, D., Russo, F., Torrieri, E., Masi, P., Villani, F., 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl. Environ. Microbiol. 72, 4663–4671.

Gardini, F., Bover-Cid, S., Tofalo, R., Belletti, N., Gatto, V., Suzzi, G., 2008. Modeling the aminogenic potential of *Enterococcus faecalis* EF37 in dry fermented sausages through chemical and molecular approaches. Appl. Environ. Microbiol. 74 (9), 2740-2750.

Gill, C. O., Penney, N., 1988. The effect of the initial gas volume to meat weight ratio on the storage life of chilled beef packaged under carbon dioxide. Meat Sci. 22, 53–63.

Gonzalez-Fernandez, C., Santos, E.M., Jaime, I., Rovira, J., 2003. Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. Food Microbiol. 20, 275-284.

Gosetti, F., Mazzucco, E., Gianotti, V., Polati, S., Gennaro, M. C., 2007. High performance liquid chromatography/tandem mass spectrometry determination of biogenic amines in typical Piedmont cheese. J. Chromatogr. A, 1149, 151-157.

Kanki, M., Yoda, T., Tsukamoto, T., Baba, E., 2007. Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. Appl. Environ. Microbiol. 73 (5), 1467-1473.

Kotzekidou, P., Bloukas, J. G., 1996. Effect of protective cultures and packaging film permeability on shelf-life of sliced vacuum- packed cooked ham. Meat Sci. 42, 333–345.

Korkeala, H., Lindroth, S., Ahvenainen, R., Alanko, T., 1987. Interrelationship between microbial numbers and other parameters in the spoilage of vacuum- packed cooked ring sausages. Int. J. Food Microbiol. 5, 311–321.

Korkeala, H., Björkroth, K.J., 1997. Microbiological spoilage and contamination of vacuum-packaged cooked sausages. J. Food Protect. 60, 724–734.

Jarmoluk, A., Pietrasik, Z., 2003. Response surface methodology study on the effects of blood plasma, microbial transglutaminase and kappa-carrageenan on pork batter gel properties. J. Food Engin. 60, 327–334.

510 Jeremiah, L. E., Gibson, L. L., Argnosa, G. C.,1994. The influence of controlled atmosphere and
511 vacuum packaging upon chilled pork keeping quality. Meat Sci. 40, 79–92.

512 Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Vidal- Carou, M. C.,
513 1996. Ion pair liquid chromatographic determination of biogenic amines in meat and meat
514 products. J. Agricul. Foods Chem. 44, 2710-2715.

515 Hernández-Jover, T., Izquierdo-Pulido, M., Veciana-Nogués, M. T., Mariné- Font, A., Vidal-
516 Carou, M. C., 1997. Biogenic amine and polyamine contents in meat and meat products. J.
517 Agricul. Food Chemistry 45, 98-102.

518 Ladero, V., Fernández, M., Calles-Enríquez, M., Sánchez-Llana, E., Cãnedo, E., Martín, M. C.,
519 Alvarez, M. A., 2012. Is the production of the biogenic amines tyramine and putrescine a
520 species-level trait in enterococci? Food Microbiol. 30, 132-138.

521 Lucas, P. M.; Blancato, V. S.; Claisse, O.; Magni, C.; Lolkema, J. S.; Lonvaud-Funel, A., 2007.
522 Agmatine deiminase pathway genes in *Lactobacillus brevis* are linked to the tyrosine
523 decarboxylation operon in a putative acid resistance locus. Microbiol. 153, 2221−2230.

524 Marcobal, A., De Las Rivas, B., Landete, J. M., Tabera, L., Muñoz, R., 2012. Tyramine and
525 phenylethylamine biosynthesis by food bacteria. Crit. Rev. Food Sci. Nutr. 52, 448-467.

526 McCabe, B. J., 1986. Dietary tyramine and other pressor amines in MAOI regiments: a review. J.
527 Am. Diet. Assoc. 86, 1059-1064.

528 Montel, M.C., Masson, F., Talon, R., 1998. Bacterial role in flavour development. Meat Sci. 49,
529 suppl. 1, s111-s123.

530 Mottram, D.S., 1998. Flavour formation in meat and meat products: a review. Food Chem. 66,
531 845-854.

Niemelä, S. I., Mentu, J., Väätänen, P., Lahiti, K., 1983. Maximum growth temperature as a diagnostic character in Enterobacteriaceae. Gram negative bacteria of medical and public health importance. INSERM, Paris, pp. 619-627.

Pearson, D., 1976. The chemical analysis of foods. Ed. Churchill Livingstone, Londra, pp. 365-401.

Pereira, A., Dionísio, L., Patarata, L., Matos, T. J. S., 2015. Effect of packaging technology on microbiological and sensory quality of a cooked blood sausage, Morcela de Arroz, from Monchique region of Portugal. Meat Sci. 101 (2015) 33–41

Pircher, A., Bauer, F., Paulsen, P., 2007. Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. Eur. Food Res. Techn. 226 (1,2), 225-231.

Ridell, J., Korkeala, H., 1997. Minimum growth temperatures of Hafnia alvei and other Enterobacteriaceae isolated from refrigerated meat determined with a temperature gradient incubator. Int. J. Food Microbiol. 35, 287-292.

Roig-Sagués, A. X., Hernández-Herrero, M., López-Sabater, E. I., Rodriguez- Jerez, J. J., Mora-Ventura, M. T., 1999. Microbiological events during the elaboration of "fuet", a Spanish ripened sausages. Relationships between the development of histidine and tyrosine decarboxylase containing bacteria and pH and water activity. Eur. Food Res. Techn. 209, 108-112.

Rossi, F., Gardini, F., Rizzotti, L., La Gioia, F., Tabanelli, G., Torriani, S., 2011. Features of the histidine decarboxylase activity of *Streptococcus thermophilus* PRI60: quantitative analysis of *hdc*A transcription and factors influencing histamine production. Appl. Environ. Microbiol. 77 (8), 2817-2822.

Samelis, J., Kakouri, A., Rementzis, J., 2000. Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4 °C. Food Microbiol. 17, 329–340.

Santos, E.M., Diez, A.M., González-Fernández, C., Rovira, J., 2005. Microbial and sensory changes in morcilla de Burgos preserved in air, vacuum and modified atmosphere packaging. Meat Sci. 71, 249–255.

Seefeldt, K. E., Weimer, B. C., 2000. Diversity of sulfur compound production in lactic acid bacteria. J. Dairy Sci. 83, 2740-2746.

Shah, P. Swiatlo, E., 2008. A multifaceted role for polyamines in bacterial pathogens. Mol. Microbiol. 68, 4−16.

Shahidi, F., Rubin, L.J., D'Sousa, L.A., 1986. Meat flavour volatiles: A review of the composition, techniques of analysis, and sensory evaluations. CRC Critl Rev. Food Sci. Nutr. 24, 141-143.

Suzzi, G., Gardini, F., 2003. Biogenic amines in dry fermented sausages: A review. Int. J. Food Microbiol. 88 (1), 41-54.

Van Straten, S., 1977. Volatile Compounds in Food, 4th ed. Central Institute for Nutrition and Food Research TNO, Zeist, The Netherlands.

von Holy, A., Holzapfel, W. H. Dykes, G. A., 1992. Bacterial populations associated with Vienna sausage packaging. Food Microbiol. 9, 45–53.

Conflict of Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Table 1: Fate of microorganisms in "Sanganel" blood sausages stored at 4 ± 2 °C for 30 days.

Microorganisms	**Days**			
	0	7	14	30
Total viable count	4.1 ± 0.2 a	7.1 ± 0.4 b	7.5 ± 0.1 b	8.5 ± 0.1c
Lactic acid bacteria	4.8 ± 0.4 a	5.3 ± 0.5 a	6.5 ± 0.3 b	8.5 ± 0.3c
Yeasts	4.0 ± 1.1	4.1 ± 1.2 a	6.0 ± 0.3 b	5.0 ± 0.3b
Moulds*	< 10	< 10	< 10	< 10
Enterococci	3.1 ± 0.5 a	3.3 ± 0.5 a	3.6 ± 0.5 a	4.8 ± 0.2b
E. *coli*	2.3 ± 0.3 a	1.7 ± 0.4 a	1.5 ± 0.5 a	1.5 ± 0.2a
Enterobacteriaceae	3.1 ± 0.8 a	5.4 ± 0.6 a	6.2 ± 0.1 a	8.2 ± 0.3b
***Pseudomonas* spp.**	3.1 ± 0.4 a	3.3 ± 0.2 a	3.1 ± 0.2 a	2.7 ± 0.5 a
CNCPC$_1$	4.1 ± 0.3 a	6.3 ± 0.5 b	6.6 ± 0.8 b	6.0 ± 1.0b
CPCPC*$_2$	< 10	< 10	< 10	< 10
Clostridia H_2S+*	< 10	< 10	< 10	< 10

Legend: Data in log CFU/g - * CFU/g; CNCPC$_1$: Coagulase Negative Catalase Positive Cocci; CCPPC$_2$: Coagulase Positive Catalase PositiveCocci; Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($p < 0.05$).

Table 3: Fate of TVB-N and biogenic amines in “Sanganel” blood sausages stored at 4 ± 2 °C for 30 days

Parameter	**Days**			
	0	**7**	**14**	**30**
TVB-N^	15.0 ± 5.2a	18.5 ± 3.5b	42.5 ± 3.8b	52.5 ± 1.2c
Histamina	5.2 ± 1.8a	4.8 ± 1.8a	7.3 ± 1.7b	10.9 ± 0.3c
Putrescine	< L.O.D.	< L.O.D.	9.1 ± 1.6a	12.5 ± 1.1b
Cadaverine	4.8 ± 1.2a	5.3 ± 1.3a	5.7 ± 1.8a	8.7 ± 1.2b
Spermine	< L.O.D.	< L.O.D.	4.7 ± 2.8a	4.9 ± 1.3a
Spermidine	< L.O.D.	< L.O.D.	7.7 ± 1.5a	7.5 ± 1.8a
Tyramine	< L.O.D.	< L.O.D.	< L.O.D.	< L.O.D.

Legend: Data TVB-N: ^Total Volatile Nitrogen mg N/100 g; Biogenic amines: mg/kg; < L.O.D.: Limit of quantitation (1.7 to 22.5 µg/L); Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($p < 0.05$).

Table 2: Fate of physico-chemical paramaters of “Sanganel” blood sausages stored at 4 ± 2 °C for 30 days

Parameter	Days			
	0	**7**	**14**	**30**
pH	6.4 ± 0.2a	6.4 ± 0.1a	6.7 ± 0.1b	6.9 ± 0.1c
Aw	0.97 ± 0.01a	0.97 ± 0.01a	0.97 ± 0.01a	0.96 ± 0.01a
Ash	3.0 ± 1.2a	3.2 ± 1.4a	3.1 ± 1.0a	3.4± 1.4a
Fat	34.5 ± 1.5a	33.7 ± 1.4a	34.0 ± 1.2a	33.7 ± 0.8a
Moisture	49.4 ± 1.5a	49.0 ± 1.8a	48.5 ± 1.2a	48.2 ± 1.2a
Protein	14.6 ± 1.3a	14.5 ± 1.8a	14.5 ± 1.5a	14.7 ± 1.5a
Sugar	trace	trace	trace	trace
L*	33.2 ± 1.0a	41.2 ± 8.1a	40.2 ± 7.1a	39.5 ± 4.3a
a*	21.0 ± 1.7a	22.3 ± 1.3a	21.2 ± 1.1a	25.4 ± 2.1a
b*	1.7 ± 0.2a	2.0 ± 0.5a	1.9. ± 1.1a	2.2. ± 0.9a

Legend: Trace: < 0.1 %; Data Ash, Fat, Moisture, Protein, Sugar g/100 g; Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($p < 0.05$).

Table 4: Gram negative strains isolated

Gram -			
Oxydase +		**Oxydase – D+**	
Strains	%	**Strains**	%
Pseudomonas putida	32	*Pantoea agglomerans**	6
Pseudomonas fluorescens	30	*Enterobacter cloacae*	9
Pseudomonas fragi	10	*Hafnia alvei*	45
Pseudomonas cepacia	13	*Citrobacter freundii*	12
Shewanella putrefaciens	13	*Klebsiella oxytoca*	10
Moraxella spp.	2	*Serratia liquefaciens*	18
Total	**100**	**Total**	**100**

Legend: * *Pantoea (Enterobacter) agglomerans*; D+: Decarboxylase positive

Table 5: Fate of volatile compounds during “Sanganel” blood sausages storaging

		0-14 day		30 day	
RT	**Compounds**	**Mean**	**(±)SD**	**Mean**	**(±)SD**
	Hydrocarbons				
1.69	Hexane	174.18	15.38a	355.44	256.33a
2.39	Octane	46.24	4.08a	67.63	30.25a
Sum		**220.42**		**423.07**	
	Aldehydes				
1.92	Acetaldehyde	371.12	32.76a	329.04	59.51a
10.07	Hexanal	8.25	0.73a	81.15	60.08b
18.74	Octanal	31.91	2.82a	104.30	80.36a
21.69	Nonanal	59.91	5.29a	313.33	98.39b
22.56	2-octenal	8.77	0.77	35.56	20.88b
27.32	2-decenal	89.13	7.87a	314.04	200.07b
28.64	Dodecanal	31.13	2.75a	78.45	66.91a
29.44	2-Undecenal	84.20	7.43a	304.35	201.33b
30.56	2,4-Decadienal	12.38	1.09a	41.19	40.74a
30.64	Tridecanal	12.08	1.07a	35.50	33.11a
Sum		**708.88**		**1,636.91**	
	Ester				
2.68	Acetic acid methyl ester	1,301.75	114.93a	1632.57	167.84b
3.4	Acetic acid ethyl ester	3,151.74	278.25a	3405.44	358.77a
3.71	Propanoic acid methyl ester	147.85	13.05a	160.96	18.53a
9.51	3-Methylbutanoic etyl ester	36.62	3.23a	51.78	21.43a
12.17	3-Methyl-1-butanol acetate	44.14	3.90a	53.35	13.01a
16.97	Hexanoic acid ethyl ester	96.62	8.53a	117.82	29.98a
20.41	2-Idroxypropanoic ethyl ester	94.60	8.35a	111.81	24.33a
21.6	Octanoic acid methyl ester	75.24	6.64a	105.39	42.63a
22.72	Octanoic ethyl ester	221.32	19.54a	314.90	132.33a
27.2	Decanoic acid ethyl ester	79.70	7.04a	134.86	78.00a
Sum		**5,249.58**		**6,088.85**	
	Alcohol				
4.39	Ethanol	3,876.32	342.23a	8,185.73	612.47b
8.14	1-Propanol	144.69	12.77a	165.20	28.99a
16.14	3-Methyl-1-butanol	237.70	20.98a	546.64	100.74b
17.63	1-Pentanol	73.28	6.47a	82.24	12.67a
19.73	2-Heptanol	102.89	9.08a	169.64	94.39a
20.66	1-Hexanol	176.73	15.60a	185.96	13.05a
23.23	1-Heptanol	23.09	2.04a	60.49	52.89a
25.51	1-Octanol	48.20	4.26a	112.84	91.41a
27.71	2-Furan Methanol	91.64	8.09a	151.36	84.44a
31.63	Safrol	86.22	7.61a	126.95	57.59a
Sum		**8,860.76**		**9,787.05**	
	Sulfur compounds				
4.74	Allyl methyl sulfur	225.59	19.92a	336.51	156.85a
12.99	Allyl sulfur	36.27	3.20a	52.77	23.33a
Sum		**261.86**		**389.28**	

	Terpenes				
6.71	α-Pinene	186.10	16.43a	190.32	5.96a
7.04	α-Fellandrene	137.78	12.16a	146.04	11.68a
8.69	Canfene	23.31	2.06a	28.78	7.72a
10.45	β-Pinene	214.59	18.95a	237.92	32.9a
11.34	α-Tuiene	515.76	45.53a	545.88	42.58a
12.73	δ-3-Carene	211.29	18.65a	198.92	17.49a
14.04	β-Myrcene	919.19	81.15a	1,009.23	127.33a
15.17	δ-Limonene	1,391.52	122.85a	1,547.50	220.58a
15.49	β-Tuiene	404.13	35.68a	416.37	17.30a
17.09	γ-Terpinene	553.08	48.83a	626.43	103.72a
17.53	β-Ocimene	111.00	9.80a	97.35	19.31a
17.98	m-Cymene	268.26	23.68a	302.57	48.51a
23.93	Copaene	260.12	22.96a	325.24	92.09a
25.32	Linalool	909.57	80.30a	1122.56	301.20
26.27	Cariofillene	785.72	69.37a	977.50	271.21a
26.44	4-Terpineol	194.71	17.19a	234.41	56.137a
28.37	Terpene	442.35	39.05a	519.28	108.79a
28.93	β-Bisabolene	209.01	18.45a	272.39	89.63a
29.51	β-Cadinene	71.32	6.30a	78.43	10.04a
31.15	Geraniol	33.86	2.99a	53.56	27.86a
Sum		**7,842.67**		**8,930.68**	
	Ketones				
2.56	2-Propanone	41.36	3.65a	52.06	15.12a
16.84	2-Penthyl Furan	33.28	2.94a	63.28	42.42a
Sum		**74.64**		**115.34**	
	Carboxylic acid				
23.51	Acetic acid	715.65	63.18a	1.820.69	550.69b
Sum		**715.65**		**1,858.01**	

Legend: Data (mean of 10 samples) expressed in µg/Kg; Sum of compounds; RT: Retention time. Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values) are not significantly differently (P< 0.05).

Table 6: Sensorial evaluation of “Sanganel” blood sausages stored 4 ± 2 °C for 30 days

Parameter	**Days**		
	0	14	30
External appearance	4.3 ± 0.5a	4.5 ± 0.5a	4.4 ± 0.3a
Slice appearance	4.5 ± 0.5a	4.5 ± 0.4a	4.5 ± 0.4a
Odour	4.5 ± 0.5a	4.4 ± 0.3a	4.4 ± 0.5a
Taste	4.8 ± 0.2a	4.4 ± 0.3a	4.5 ± 0.3a
Unpleasant odour*	1.3 ± 0.5a	1.4 ± 0.7a	1.4 ± 0.3a

Legend: 5 = excellent, 4 = good, 3 = acceptable, 2 = fair, 1 = unacceptable; *1 corresponded to the absence of these odours or odours of a minimum intensity and 5 corresponded to odours of a maximum intensity; Data represent the means ± standard deviations of the total samples; Mean with the same letters within the same lane (following the values) are not significantly differently ($p < 0.05$).

-